D1

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 37/36, C07K 7/08, 15/00 — A1

(11) International Publication Number: **WO 95/00166**

(43) International Publication Date: 5 January 1995 (05.01.95)

(21) International Application Number: PCT/GB94/01309

(22) International Filing Date: 17 June 1994 (17.06.94)

(30) Priority Data:
08/079,637 18 June 1993 (18.06.93) US

(71) Applicants *(for all designated States except US)*: YISSUM RESEARCH DEVELOPMENT COMPANY [IL/IL]; The Hebrew University of Jerusalem, 46 Jabotinsky Street, 91999 Jerusalem (IL). GOLDIN, Douglas, Michael [GB/GB]; 14 South Square, Gray's Inn, London WC1R 5LX (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: BAB, Itai [IL/IL]; 26 Hateena Street, Carmei Joseph, 99797 M.P. Ayalon (IL). MUHLRAD, Andras [IL/IL]; 29/A Neve Shaanan Street, 93798 Jerusalem (IL). CHOREV, Michael [IL/IL]; 134/4 Feinstein Street, 93812 Jerusalem (IL). SHTEYER, Arie [IL/IL]; 37 Ha'arazim Street, 90805 Mevasseret Zion (IL). SLAVIN, Shimon [IL/IL]; Ein Kerem 402, 95744 Jerusalem (IL). MANSUR, Nura [IL/IL]; 3/24 Giborei Israel Street, 72462 Ramla (IL). GUREVITCH, Olga [IL/IL]; 17 Etzel Street, 97853 Jerusalem (IL).

(74) Agents: GOLDIN, Douglas, Michael et al.; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5LX (GB).

(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: PHARMACEUTICAL COMPOSITIONS FOR STIMULATING RECONSTRUCTION OF HEMOPOIETIC MICROENVIRONMENT

(57) Abstract

The invention relates to a pharmaceutical composition for stimulating reconstruction of hemopoietic microenvironment comprising a therapeutically effective amount of a peptide having an amino acide sequence Ala-Leu-Lys-Arg-Gln-Gly-Arg-Thr-Leu-Try-Gly-Phe-Gly-Gly- (OGP), or conservative amino acid substitution or deletion thereof, or an oligopeptide having molecular weight of from 200 to 2,000 wherein the four C-terminal amino acid residues are identical with those of the said OGP, with the penultimate Gly optionally replaced by His, or a mixture thereof and a pharmaceutically acceptable carrier. A method of stimulating reconstruction of hemopoietic microenvironment in a mammal which comprises administering to said mammal a therapeutically effective amount of a peptide having an amino acid sequence Ala-Leu-Lys-Arg-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-Gly-Gly (OGP), or conservative amino acid substitution or deletion thereof, or an oligopeptide having molecular weight of from 200 to 2,000 wherein the four C-terminal amino acid residues are identical with those of said OGP, with the penultimates Gly optionally replaced by His, or a mixture thereof.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CS	Czechoslovakia
CZ	Czech Republic
DE	Germany
DK	Denmark
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgystan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakhstan
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SI	Slovenia
SK	Slovakia
SN	Senegal
TD	Chad
TG	Togo
TJ	Tajikistan
TT	Trinidad and Tobago
UA	Ukraine
US	United States of America
UZ	Uzbekistan
VN	Viet Nam

PHARMACEUTICAL COMPOSITIONS FOR STIMULATING RECONSTRUCTION OF HEMOPOIETIC MICROENVIRONMENT

Field of the Invention

The invention relates to use of osteogenic growth oligopeptides and fragments thereof for stimulating reconstruction of hemopoietic microenvironment.

Background of the Invention

An osteogenic growth polypeptide (OGP) which possesses stimulatory activity on osteoblastic and fibroblastic cells is known, for example from European Patent Applications Nos. 0 349 048 and 0 384 731. This 14-amino acid osteogenic growth polypeptide has been found to be identical with the C-terminus of histone H4. A synthetic 14-mer osteogenic growth polypeptide (sOGP), identical in structure with the native molecule, has been shown to be a potent stimulator of proliferation of osteoblastic cell alkaline phosphatase activity. When injected <u>in vivo</u> to rats, at very small doses, the synthetic osteogenic growth polypeptide increased bone formation and trabecular bone mass. Several biologically active fragments of OGP, whether derived from the native OGP or synthetic, have also been shown to also possess the osteogenic activity. Such osteogenic oligopeptides form the subject of PCT/GB 94/00416.

It has now been found that OGP, as well as the said biologically active fragments thereof, whether native or synthetic, are capable of enhancing the engraftment of bone

marrow transplant and stimulate reconstruction of the hemopoietic microenvironment.

Bone marrow transplantation (BMT) is progressively and rapidly becoming the treatment of choice in instances of haematological malignancies such as lymphomas, Hodgkin's disease and acute leukaemia as well as solid cancers, in particular melanoma and breast cancer. Potentially, with improved methods, BMT can also be used for treating other catastrophic diseases like AIDS, aplastic anaemia and autoimmune disorders. The aim of all BMT is to replace the host hemopoietic stem cells, totipotent and pluripotent, injured by chemotherapy, radiation or disease. These stem cells can replicate repeatedly and differentiate to give rise to the whole variety of cells present in blood-erythrocytes, monocytes and neutrophils. Resident macrophages and osteoclasts are also derived from hemopoietic totipotent stem cells. As the stem cells differentiate, they commit themselves more and more to a particular lineage until they can form only one kind of the above cells.

The most common way currently available for acquiring enough stem cells for transplantation is to extract one litre or more of marrow tissue from multiple site in the donor's bones with needle and syringe, an involved process that usually requires general anaesthesia. The donors of allogenic BMT are usually siblings whose tissue types are compatible and sometimes unrelated donors who are matched to the recipient by HLA typing. Autologous transplants, that eliminate the need for

HLA matching, may be used in patients undergoing ablative chemoradiotherapy for the eradication of solid tumours. Autologous stem cells may also be obtained from the umbilical cord blood at birth and stored for future administration.

After transplantation and prior to the establishment of a donor-derived functioning marrow the patients hosting BMT present with a transient marked pancytopenia that exposes them to infections. The incidence of bacterial and fungal infection correlates with both the severity and duration of pancytopenia [Slavin S. and Nagler A., (1992) Transplantation]. The recipient must therefore receive a steady supply of fresh red cells, platelets and antibiotics for several weeks until the transplanted stem cells begin producing large quantities of mature blood elements. In instances of allogenic BMT the recipient immune system must be sufficiently suppressed so that it will not reject the transplanted stem cells. At the same time, the transplanted donor's immune system may give rise to graft versus host disease (GVHD) and cause lethal tissue and organ damage. All these considerations dictate prolonged and expensive hospitalization.

BMT could be such more effective if a way could be found to accelerate the process of engraftment, enhance marrow reconstruction, reduce medical hurdles and shorten the hospitalization period and the incidence of infection, morbidity and mortality [Gabrilove J.L., et al. (1988) N. Engl. J. Med. 318:1414]. The currently available clinical (experimental) treatment for stimulating post-BMT marrow

reconstruction consists mainly of the administration of recombinant human granulocyte colony stimulating factor (rhG-CSF) and/or recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF) [Blazar R. B., et al. (1989) Blood 74:2264). These cytokines affect directly the proliferation of transplanted pluripotent cells already committed to the while-cell lineages [Vellenga E., et al (1987) Leukaemia 1:584] and consequently decrease the time to leucocyte and neutrophil recovery.

There are, however, some major concerns regarding the therapeutic use of rhG-CSF and rhGM-CSF. tumours and leukaemic cells posses normal receptors for these cytokines [Vellenga E., et al. ibid.] and their administration can increase relapse rates by enhancing the proliferation of residual host tumour cells. Another concern about using CSFs in the setting of BMT is that the CSFs, by stimulating the proliferation of relatively committed cells with no capacity for self renewal, deplete progenitor cell number [Slavin S. and Nagler A. ibid.]. For a similar reason, the CSFs fail to support erythropoiesis and platelet formation.

Polypeptides that support haemopoiesis may prove useful in other ways as well. Some investigators have found that adding stem cells from the peripheral blood to those from the bone marrow significantly increases the rate of engraftment extracting sufficient numbers of stem cells from peripheral blood is a complicated procedure. Administrating such polypeptides to donors to increase the number of stem cells in

the blood will improve the feasibility of transplanting stem cells from peripheral blood [Golde D.W., (1991) Sci. Am. December:36].

A prerequisite for haemopoiesis and therefore successful BMT is the presence of functional stromal cells and tissue that comprise the hemopoietic microenvironment, determine the homing of the injected stem cells from the circulation to the bone marrow and support haemopoiesis [Watson J.D and McKenna H. J. (1992) Int. J. Cell Cloning 10:144]. Marrow derived stromal tissue also provide the conditions to sustain stem cells in in vitro long-term bone marrow cultures. At present this technology suffices to keep stem cells alive. Adding the appropriate hemopoietic polypeptides to these cultures may help expand the stem cell population in vitro, this providing increased numbers of these cells for transplantation. The combined in-vitro in-vivo approach may provide the basis for a forward-looking strategy for (i) obtaining small stem cell preparation from donors' blood or marrow and (ii) healthy individuals to have their stem cells stored for a time when the cells might be needed to treat a serious disease, thus bypassing the complexity associated with the use of allogenic BMT.

It would therefore be of therapeutic importance to find a small peptides that stimulate post BMT memopoietic reconstruction by enhancing in-vivo and/or in vitro the hemopoietic microenvironment of which fibrous tissue, bone and bone cells are important components. Such peptides may also support

haemopoiesis in spontaneous occurring or induced myelosuppression condition that do not necessarily involved BMT.

Preablation therapy using molecules with an OGP-like activity, such as OGP and its said therapeutically active fragments is likely to enhance the hemopoietic microenvironment and consequently stimulate haemopoiesis at the noncommitted stem cell level avoiding the stem cell depiction and white cell discrimination.

Summary of the Invention

The invention relates to pharmaceutical compositions for the enhancement of engraftment of bone marrow transplants and/or stimulation of reconstruction of hemopoietic microenvironment comprising a therapeutically effective amount of biochemically pure or synthetic osteogenic growth polypeptide (OGP) or therapeutically active fragments thereof.

The invention also relates to use of biochemically pure or synthetic OGP or therapeutically active fragments thereof in the preparation of pharmaceutical compositions for the enhancement of engraftment of bone marrow transplant and stimulation of reconstruction of hemopoietic microenvironment.

The invention further relates to a method simulating reconstruction of hemopoietic microenvironment in a mammal which comprises administering to said mammal a therapeutically effective amount of biochemically pure or synthetic OGP or

therapeutically active fragments thereof.

Brief Description of the Figures

Figure 1 shows a dose dependent effect of pretreatment with OGP(1-14) on the total number of femoral marrow cells in mice after combined ablative radiotherapy/BMT. sOGP (synthetic OGP) at the indicated dose was injected sub-cutaneously daily for 12 days to female C57 Black mice. On day 8 after the onset of sOGP treatment the mice were subjected to 900 rad X-ray irradiation followed by intravenous administration of 10^5 syngeneic unselected marrow cells. On day 14 after the onset of treatment the mice were sacrificed and the femoral marrow washed out into phosphate buffered saline. A single cell suspension was prepared by drawing the preparation several times through graded needles. Cell counts were carried out in a haemocytometer. C-control mice given phosphate buffered saline only. Data are mean± SEM obtained in at least seven mice per condition.

Figure 2 shows a dose dependent effect of pretreatment with OGP(10-14) on the total number of femoral marrow cells in mice after combined ablative radiotherapy/BMT. OGP(10-14) at the indicated dose was injected subcutaneously daily for 12 days to female C57 BL mice. On day 8 after the onset of OGP(10-14) treatment the mice were subjected to 900

Rad X-ray irradiation followed by intravenous administration of 10^5 syngeneic unselected marrow cells. On day fourteen after the onset of treatment the mice were sacrificed and the femoral marrow washed out into phosphate buffered saline. A single cell suspension was prepared by drawing the preparation several times through graded needles. Cell counts were carried out in a haemocytometer. C-control mice given phosphate buffered saline only. Data are mean ± SE obtained in at least seven mice per condition.

Detailed Description of the Invention

OGP is a 14-residue polypeptide identified from regenerating bone marrow which has been shown to stimulate the proliferation and alkaline phosphatase activity to osteoblastic and fibroblastic cells in vivo. The amino acid sequence of OGP is as follows:

Ala-Leu-Lys-Arg-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-Gly-Gly

Synthetic OGP, with an identical amino acid sequence and biological activity has been prepared by standard solid phase methodology.

In addition to OGP, it has been found that fragments derived therefrom, or their synthetic analogues, retain the stimulatory activity on osteoblastic and fibroblastic cells, and consequently on bone formation. Such osteogenic oligopeptides form the subject of PCT/GB 94/00416.

Preferred oligopeptides according to said PCT patent application the invention are oligopeptides wherein the five C-terminal amino acid residues are the five amino acid residues of the said OGP amino acid sequence.

The inventors have now surprisingly found that OGP, as well as its said osteogenic oligopeptides, and pharmaceutical compositions containing them, may be used for stimulation of hemopoietic microenvironment. As such, the uses of OGP and its variants include the following:

1. Accelerate the engraftment of bone marrow transplants.
2. Enhance proliferation of transplanted stem cells and thus increase the availability of all types or hemopoietic cells including erythrocytes and platelets, thus relieving the need for supporting the host with these cells for at least several weeks.
3. Enhance the stromal hemopoietic microenvironment by increasing the stromal cell number and/or expression of stromal cell derived factors that support haemopoiesis.
4. Enhance the hemopoietic stem cell expression of receptors to factors that support haemopoiesis.
5. Enhance the "homing" of intravenously administered bone marrow transplants to the host bone marrow.
6. Enhance the restoration of blood cellularity after BMT.
7. Enable successful transplantation using reduced cell number, thus decreasing the number of (multiple) marrow extractions from donors, and enabling the use of transplants as small as 10-15 ml (instead of 1000ml).
8. Increase the number of hemopoietic totipotent and/or

pluripotent stem cells in the donor peripheral blood, thus improving the feasibility of transplanting stem cells from peripheral blood.

9. Increase the number of hemopoietic stem cells in vitro in long-term bone marrow cultures for use as transplants and also provide for a method of inhibiting growth of tumour cells in allografts from leukaemia patients.

10. Enhance the endogenous restoration of marrow and blood cellularity after chemo- and/or radiotherapy.

11. Enhance the restoration of population of resident macrophages after BMT or after chemo-and/or radiotherapy.

The invention therefore relates to pharmaceutical compositions for stimulating reconstruction of hemopietic microenvironment, particularly for enhancing the engraftment of bone marrow transplants, as well as any of the above mentioned uses, comprising as active ingredient a therapeutically effective amount of OGP or oligopeptides of molecular weight of 200 to 2000 in which the four C-terminal amino acids are identical with those of said OGP, with the penultimate Gly optionally replaced by His or mixtures thereof.

Biochemically pure OGP and said oligopeptides can be prepared by the methods described in European Patent Application No. 0 384 731 and PCT/GB 94/00416.

Preferred oligopeptides are biochemically pure oligopeptides comprising the following amino acid sequences:

Tyr-Gly-Phe-Gly-Gly;

Tyr-Gly-Phe-His-Gly;

Ac-Met-Tyr-Gly-Phe-Gly-Gly; and

Gly-Phe-Gly-Gly

The invention further relates to use of OGP or oligopeptides or molecular weight of 200 to 2000 in which the C-terminal amino acid residues are identical with those of OGP, with the penultimate Gly optionally replaced by His or mixtures thereof in the preparation of pharmaceutical compositions for simulating the reconstruction of hemopoietic microenvironment.

The invention also relates to a method of simulating reconstruction of hemopoietic microenvironment in a mammal which comprises administering to said mammal a therapeutically effective amount of a peptide having an amino acid sequence Ala-Leu-Lys-Arg-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-Gly-Gly- (OGP), or conservative amino acid substitution or deletion thereof, or an oligopeptide having molecular weight of from 200 to 2,000 wherein the four C-terminal amino acid residues are identical with those of OGP, with the penultimate Gly optionally replaced by His, or a mixture thereof, or which comprises administering to said mammal the pharmaceutical composition according to the invention.

As shown in the following Examples and accompanying drawings, OGP and OGP(10-14) have a dose-dependent stimulatory effect on the number of post irradiation/post transplantation effect on the number of post irradiation/post transplantation femoral

bone marrow cells. Thus, OGP and "OGP-like" oligopeptides have been shown to enhance the engraftment of bone marrow transplants.

The magnitude of a therapeutic dose of a polypeptide of the invention will of course vary with the group of patients (age, sex, etc.), the nature of the condition to be treated and with the particular peptide employed and its route of administration. In any case the therapeutic dose will be determined by the attending physician.

Any suitable route of administration may be employed for providing a mammal, especially a human, with an effective dosage of a peptide of this invention. Intravenous and oral administration may be preferred.

The pharmaceutical compositions of the invention can be prepared in dosage units forms. The dosage forms may also include sustained release devices. The compositions may be prepared by any of the methods well-known in the art of pharmacy.

The pharmaceutical compositions of the invention comprise as active ingredient an oligopeptide of this invention or a mixture of such oligopeptides in a pharmaceutically acceptable carrier, excipient or stabilizer, and optionally other therapeutic constituents. Acceptable carriers, and optionally other therapeutic constituents. Acceptable carriers, excipients, adjuvants or diluents are non-toxic to recipients

at the dosages and concentrations employed, and include buffers, such as phosphate buffered saline and like physiologically acceptable buffers, and more generally all suitable carriers, excipients, adjuvants and siluents known in the art.

EXAMPLES

Example 1 - Effect of sOGP on engraftment of bone marrow transplant

Method

sOGP in phosphate buffered saline was administered to female C57 Black Mice, weight 25 grams, by subcutaneous injections of 100 μl given once daily, for 12 days. The daily dose ranged from 0.0005 to 5 nmol per mouse. The control animals received phosphate buffered saline only. On day 8 after the onset of sOGP treatment the mice received total body X-ray irradiation consisting of a single 900 rad dose using a ^{60}Co source (Picker C-9, 102.5 rad/min). This was followed immediately by an intravenous injection of 10^5 unselected syngeneic bone marrow cells. The animals were killed 14 days after the onset of sOGP treatment, both femurs were dissected out and their epiphyseal ends removed. The bone marrow was washed out completely in to phosphate buffered saline, A single cell suspension was prepared by drawing the preparation several times through graded syringe needles and the cells were counted in a haemocytometer.

Results

Fig 3. shows a stimulatory effect of the sOGP on the numbers of

post irradiation/post transplantation femoral bone marrow cells. This effect was characterized by a dose response relationship without any influence at the lowest dose and statistically significant two-fold increase over control of cell counts in mice receiving the two highest doses.

Example 2 - Effect of OGP(10-14) on enfragment of bone marrow transplant

Method

OGP(10-14) (Tyr-Gly-Phe-Gly-Gly) in phosphate buffered saline was administered to female CV57 Black Mice, weighing 25 grams, by subcutaneous injections of 10 μ1 given once daily, for 12 days. The daily dose ranged from 0.001 to 10 nmol per mouse. the control animals received phosphate buffered saline only. On day 8 after the onset of OGP(10-14) treatment the mice received total body X-ray irradiation consisting of a single 900 rad dose using a ^{60}Co source (Picker C-9, 102.5 rad/min). this was followed immediately by an intravenous injection of 10^5 unselected syngeneic bone marrow cells. The animals were sacrificed 14 days after the onset of OGP(10-14) treatment, both femurs were dissected out and their epiphyseal ends removed. The bone marrow was washed out completely, into phosphate buffered saline. A single cell suspension was prepared by drawing the preparation several times through graded syringe needles and the cells were counted in a haemocytometer.

Results

Fig. 2 shows a stimulatory effect of the OGP(10-14) on the

number of post irradiation/post transplantation femoral bone marrow cells. This effect was characterized by a dose response relationship without any influence at the lowest dose and statistically significant two-fold increase over control of cell counts in mice receiving the three highest doses.

CLAIMS:

1. A pharmaceutical composition for stimulating reconstruction of hemopoietic microenvironment comprising a therapeutically effective amount of a peptide having an amino acid sequence Ala-Leu-Lys-Arg-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-Gly-Gly (OGP), or conservative amino acid substitution or deletion thereof, or an oligopeptide having molecular weight of from 200 to 2,000 wherein the four C-terminal amino acid residues are identical with those of the said OGP, with the penultimate Gly optionally replaced by His, or a mixture thereof and a pharmaceutically acceptable carrier.

2. A pharmaceutical composition according to claim 1 for stimulating hemopoietic reconstruction after bone marrow transplantation.

3. A pharmaceutical composition according to claim 1 or claim 2 comprising a therapeutically effective amount of OGP.

4. A pharmaceutical composition according to claim 1 or claim 2 comprising a therapeutically effective amount of an oligopeptide having a molecular weight of from 200 to 2,000 wherein the four C-terminal amino acid residues are identical with those of said OGP, with the penultimate Gly optionally replaced by His.

5. A pharmaceutical composition according to claim 4 wherein said oligopeptide comprises the amino acid sequence: Tyr-Gly-Phe-His-Gly.

6. A pharmaceutical composition according to claim 5 wherein said oligopeptide is Tyr-Gly-Phe-His-Gly.

7. A pharmaceutical composition according to claim 4 wherein said oligopeptide Ac-Met-Tyr-Gly-Phe-Gly-Gly.

8. A pharmaceutical composition according to claim 4 wherein said oligopeptide comprises the amino acid sequence: Tyr-Gly-Phe-Gly-Gly.

9. A pharmaceutical composition according to claim 4 wherein said oligopeptide is Gly-Phe-Gly-Gly.

10. A pharmaceutical composition according to any one of claims 1 to 9 further comprising a pharmaceutically acceptable carrier, excipient, adjuvant or diluent.

11. Use of a peptide having an amino acid sequence Ala-Leu-Lys-Arg-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-Phe-Gly-Gly (OGP), or conservative amino acid substitution or deletion thereof, or an oligopeptide having molecular weight of from 200 to 2,000 wherein the four C-terminal amino acid residues are identical with those of said OGP, with the penultimate Gly optionally replaced by His, in the manufacture of a medicament for stimulating reconstruction of hemopoietic mirconenvironment.

12. Use according to claim 11 for the preparation of a medicament for the acceleration of engraftment of bone marrow transplants, enhancing proliferation of transplanted stem cells, enhancing the stromal hemopoietic microenvironment, enhancing the hemopoietic stem cell expression of receptors to factors that support haemopoiesis, enhancing the "homing" of intravenously administered bone marrow transplants to the host bone marrow, enhancing the restoration of blood cellularity after bone marrow transplantation, enabling successful

transplantation using reduced cell number, increasing the number of hemopoietic totipotent and/or pluripotent stem cells in the donor peripheral blood, increasing the number of hemopoietic stem cells in vitro in long-term bone marrow cultures for use as transplants enhancing the endogenous restoration of marrow and blood cellularity after chemo- and/or radiotherapy and enhancing the restoration of population of resident macrophages after bone marrow transplantation or after chemo- and/or radiotherapy.

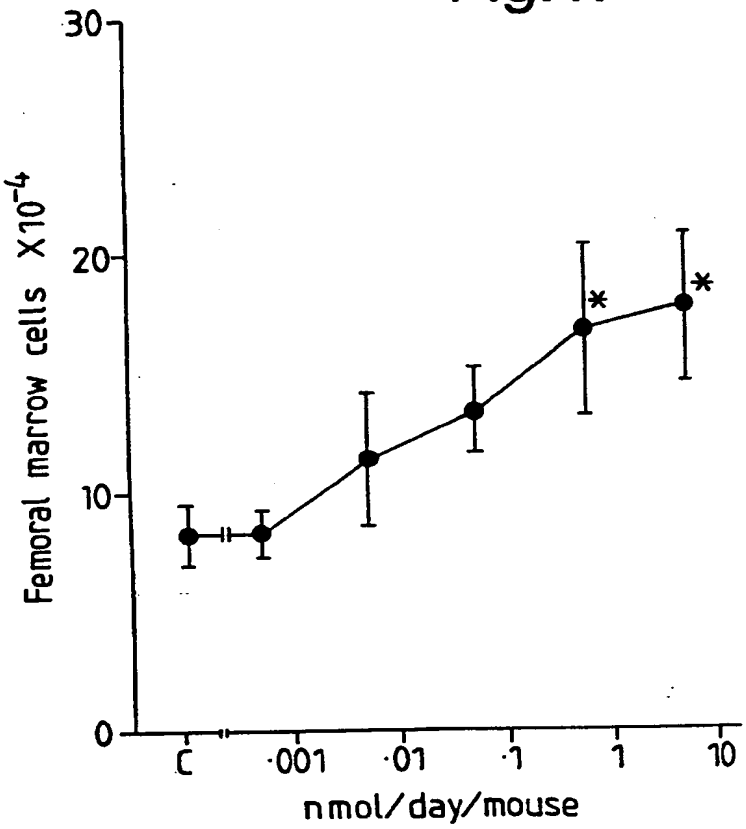
13. A method of stimulating reconstruction of hemopoietic microenvironment in a mammal which comprises administering to said mammal a therapeutically effective amount of a peptide having an amino acid sequence Ala-Leu-Lys-Arg-Gln-Gly-Arg-Thr-Ley-Tyr-Gly-Phe-Gly-Gly (OGP), or conservative amino acid substitution or deletion thereof, or an oligopeptide having molecular weight of from 200 to 2,000 wherein the four C-terminal amino acid residues are identical with those of said OGP, with the penultimate Gly optionally replaced by His, or a mixture thereof.

14. A method of stimulating reconstruction of hemopoietic micro-environment in a mammal which comprises administering to said mammal the pharmaceutical composition according to any one of claims 1 to 10.

15. Use according to claim 11 or 12 wherein the peptide or oligopeptide is as defined in any one of claims 4 to 9.

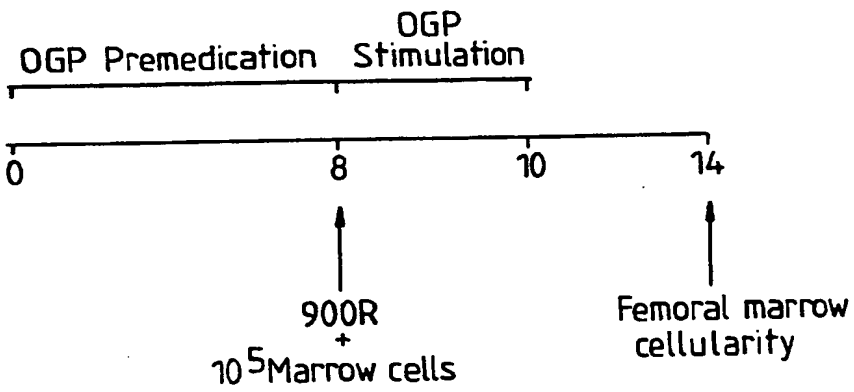
16. A method according to claim 13 wherein the peptide or oligopeptide is as defined in any one of claims 4 to 9.

Fig.1.



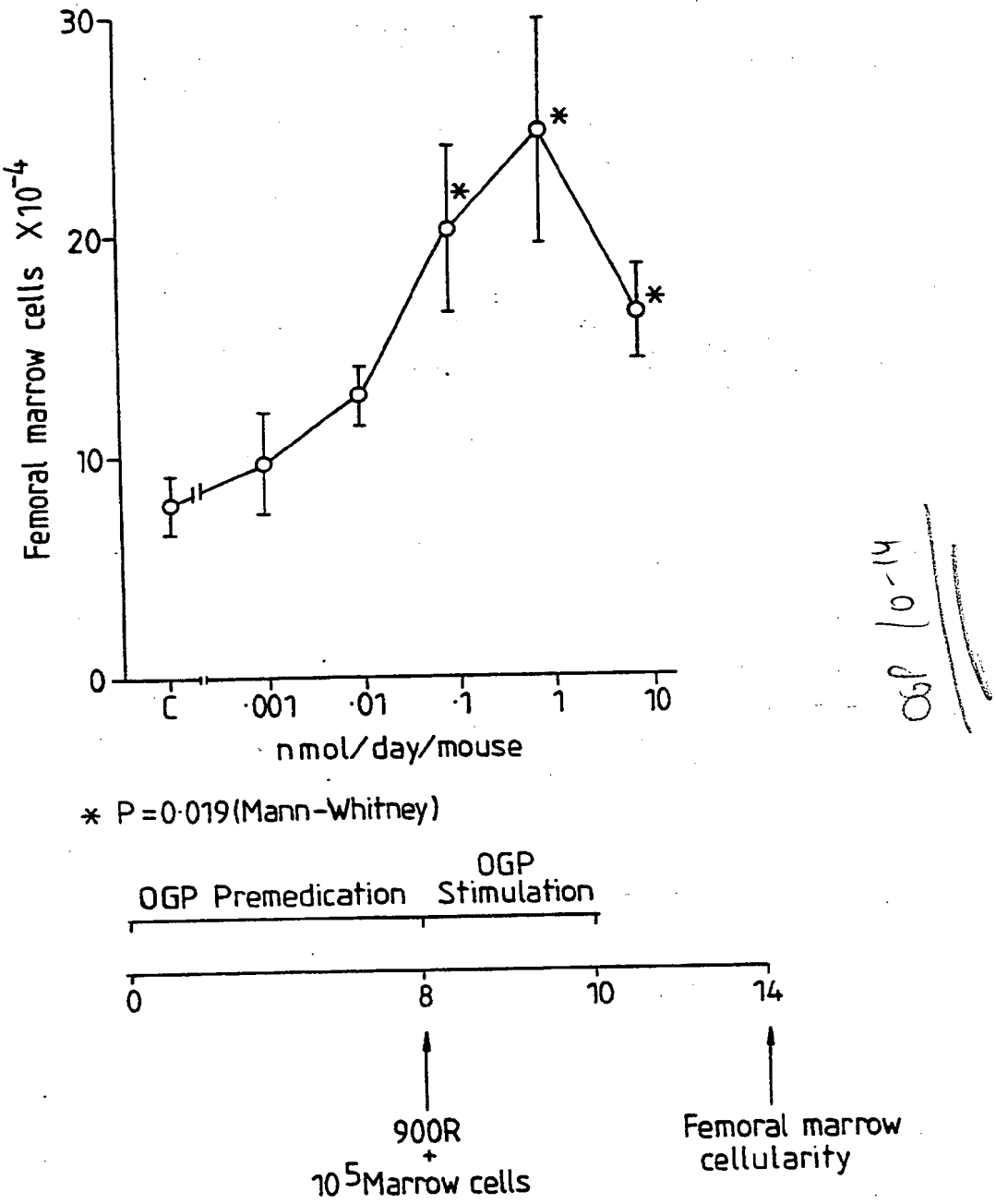
OGR

* P=0·019(Mann-Whitney)



C57 BL ♀ MICE

Fig.2.



OGP 10-14

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 94/01309

A. CLASSIFICATION OF SUBJECT MATTER

A 61 K 37/36,C 07 K 7/08,C 07 K 15/00

According to International Patent Classification (IPC) or to both national classification and IPC 5

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A 61 K,C 07 K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP, A2, 0 384 731 (MERCK & CO.INC.) 29 August 1990 (29.08.90), claims 1-3,6,8,10.	1-10

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

15 September 1994

Date of mailing of the international search report

13.10.94

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

BÖHM e.h.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 94/01309

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 13,14,16
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 13,14,16 are directed to a method of treatment of the human or animal body (Rule 39.1(iv)PCT) the search has been carried out and based on the alleged effects of the compounds.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANHANG

zum internationalen Recherchenbericht über die internationale Patentanmeldung Nr.

ANNEX

to the International Search Report to the International Patent Application No.

ANNEXE

au rapport de recherche international relatif á la demande de brevet international n°

PCT/GB 94/01309 SAE 91663

In diesem Anhang sind die Mitglieder der Patentfamilien der im obengenannten internationalen Recherchenbericht angeführten Patentdokumente angegeben. Diese Angaben dienen nur zur Unterrichtung und erfolgen ohne Gewähr.

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The Office is in no way liable for these particulars which are given merely for the purpose of information.

La présente annexe indique les membres de la famille de brevets relatifs aux documents de brevets cités dans le rapport de recherche international visée ci-dessus. Les renseignements fournis sont donnés á titre indicatif et n'engagent pas la responsibilité de l'Office.

Im Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
EP A2 384731	29-08-90	CA AA 2010660	23-08-90
		EP A3 384731	26-06-91
		JP A2 2282396	19-11-90